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AB Three classes of multigene family-encoded receptors enable NK cells to discriminate between polymorphic MHC class I molecules: Ly-49 homodimers, CD94/NKG2 heterodimers and the killer cell inhibitory receptors (KIR). Of these, CD94/NKG2 has been characterized in both rodents and humans. In contrast, Ly-49 family members have hitherto been found only in rodents, and KIR molecules only in the human. In this report, we describe a human cDNA, termed Ly-49L, that constitutes the first human member of the Ly-49 multi-gene family. Compared with rodent Ly-49 molecules, the Ly-49L sequence contains a premature stop codon and predicts a truncated protein that lacks the distal part of a C-terminal lectin domain. Evidence is presented that the premature stop codon results from incomplete excision of the intron between the first two lectin domain exons. Splice variants predicting a full-size Ly-49L protein were not detected. As demonstrated by Northern blot analysis, Ly-49L was transcribed by IL-2-activated NK cells, but not by freshly isolated B or T cells. PCR screening of a 22-clone yeast artificial chromosome contig localized the LY49L locus to the human NK gene complex on chromosome 12p12-p13. Southern blot analysis of genomic DNA showed a simple pattern with a full-length Ly-49L probe at low stringency hybridization conditions, suggesting that Ly-49L may be the only human member of the Ly-49 multigene family.